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Genome composition and tandemly repetitive sequence at some centromeres in the lizard *Podarcis s. sicula* Raf.

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Abstract

A detailed study on the genome of the lizard *P. sicula* has been carried out using restriction enzyme analysis followed by identification and cloning of a repetitive DNA fraction. The results show that *P. sicula* generally possesses a quite homogeneous genome composition, with a single tandemly repetitive sequence family that is easily visualized after digestion of genomic DNA with Taq I. The cloned repeating unit of this satellite (260 bp) has been designed pLCSI. *In-situ* hybridisation shows that this satellite is localized in the centromeric region. Dot blot experiments show that sequences similar to pLCSI are present in other species of the same family of lizards.

Introduction

Recent research suggests the existence of considerable differences between DNA composition of heterotherm vertebrates (above all anamniotes) and that of homeotherms (Thiery *et al.*, 1976; Birstein, 1982; Morescalchi & Olmo, 1982; Bernardi *et al.*, 1985). Such differences especially involve the presence or absence of particular types of middle and highly repeated DNA (e.g. the DNA of birds and mammals possesses certain guanine + cytosine rich sequences that seem to be absent in other vertebrates, Bernardi *et al.*, 1985).

Reptiles occupy an intermediate position between these two groups of vertebrates and some evidence suggests that also their genome presents intermediate characteristics between that of heterotherms and that of homeotherms (Olmo, 1986). However, studies on reptilian genomes, and on the genomes of other heterotherms, are still extremely limited, in contrast to those on the genomes of mammals, which are numerous and thorough.

In this paper we report the initial results of a study on the genome of a lizard, *Podarcis sicula*, obtained through restriction enzyme analysis, preparation of a genomic library, identification and cloning of particular repetitive DNA fractions, and determination of their localization on chromosomes. This study is of further interest because, in this species, intraspecific differences have been observed in the quantity and distribution of constitutive heterochromatin (Olmo *et al.*, 1986). These differences have been linked to variation in specific fractions of highly repetitive DNA (Olmo *et al.*, 1987.)

Materials and methods

Animals. Specimens of *Podarcis s.sicula* (Rafinesque) were collected from Regi Lagni and Sapri

(Salerno, Italy). Individuals of *Podarcis muralis* (Laurenti) were kindly provided by Dr. V. Caputo and collected near San Vincenzo (Campobasso Italy). *P. wagleriana* (Gistel) was collected near Porto Palo (Siracusa Italy) and Primo Sole (Catania Italy) and kindly provided by Dr M. Capula. *Lacerta viridis* (Laurenti), *Lacerta dugesii* (Milne-Edwards) and *Takydromus sexlineatus* (Daudin) were purchased from Drs. De Rover (Holland) and the precise locality is unknown.

Materials. Restriction enzymes were obtained from New England Biolabs, Bethesda Research Laboratories Ltd., T4 DNA ligase, DNA polymerase and Klenow fragment were obtained from Boehringer-Mannheim; all enzymes were used as suggested from the suppliers. Labeled nucleotides (^{32}p-dCTP and ^{3}H-dNTPs) were from Amersham International. The labelling was carried out under conditions specified by Amersham International.

DNA extraction. Livers and testis of lizards were homogenized in cold saline/EDTA (0.15 M NaCL, 0.1 M EDTA, pH 8.0) and the DNA was isolated according to Jeffreys and Flavell (1977).

Cytological preparation. Mitotic plates for restriction enzymes analysis and *in-situ* hybridisation were prepared according to the technique previously described (Olmo *et al.*, 1986).

Restriction enzyme analysis. DNA of *P. sicula* was analized by digestion with several restriction enzymes: Alu I, Ava I, Bam HI, Bgl II, Cfo I, Cla I, Dra I, Eco RI, Eco RII, Eco RV, Hae III, Hind III, Hinf I, Hpa II, Mbo I, Msp I, Pvu II, Sau3A I, Taq I, Xba I. Each enzyme was diluted in its specific reaction mixture together with the DNA (5 μg, 2 units/μg) and incubated for 3 h at 37 °C. Digested DNA was run on a 1,2% agarose gel. The electrophoresis buffer was 1 × TBE. Chromosomes spreads were digested for 12 h with 100 units of a tested restriction enzyme diluted in 100 μl assay mixture (Miller *et al.*, 1984).

Construction of pLCSI. Total DNA (100 μg) of *P. sicula* was digested with Taq I enzyme (2 units/μg) for three h at 37 C. Taq I satellite monomer (260 bp) was extracted from a low melting agarose gel and ligated to Acc I cleaved pUC 18. After transformation of E. coli JM 83 cells, ampicillin resistent recombinants were selected on X-gal nutrient plates. The recombinant clones were analysed by agarose gel electrophoresis and Southern blotting.

In situ *hybridisation.* Hybridization of ^{3}H-labeled DNA probe to mitotic chromosomes was performed as described by Gall *et al.* (1971). Mitotic preparations were pretreated with RNase A (100 μg/ml in 2 × SSC) for two hours at room temperature. Chromosomal DNA was denaturated by boiling. Each slide received 30 μl of hybridisation mixture containing 50% formamide, 2 × SSC and 400,000 cpm ^{3}H-labelled DNA of pLCS1. Slides were then dipped in Ilford K2 emulsion diluted 1 : 1 with water, and left to expose in the dark for periods of 15–20 days at 4 °C. After development, the slides were stained with Giemsa.

Southern transfer and hybridization. Restriction enzyme digested DNA (10 μg) was separated on a 1.2% agarose gel and transferred to a nitrocellulose filter according to the method of Southern (1975). Filters were pre-hybridised at 68 °C in plastic bags containing 4% SSC, 0.1% SDS, 2% Denhardt's mix for 6 h (Denhardt, 1966). Hybridisation was carried out at 60 °C in the above solution containing 0.1 μg of ^{32}p nick translated pLCSl DNA with a specific activity of 1.5×10^{7} cpm/μg. The hybridised filters were washed in 1 × SSC, 0.1% SDS, followed by 0.1 × SSC, 0.1% SDS at 55 °C.

Dot blots. Increasing volumes of DNA solutions were slowly applied to nitrocellulose filters. The DNA was denaturated in 0.2 N NaOH, neutralized with 1.5 M Tris, 1 M NaCl pH and the filters baked for 10 h at 60 °C. Hybridisation was carried out as described above.

Results

Twelve of the 20 restriction enzymes tested cut the DNA of *P. sicula* quite extensively (Fig. 1). One group of five enzymes cut the DNA most extensively, leaving very few high molecular weight DNA fragments and a major concentration of low molecular weight fragments. These enzymes were Alu I (AGCT), Hae III (GGCC), Hinf I (GANTC), Mbo I (GATC), Sau3A I (GATC). A second group of four enzymes produced a fairly even distribution of fragments throughout the gel, leaving many more large fragments. These enzymes included Dra I (TTTAAA), Ava I (CpyCGpuG), Pvu II (CAGCTG) and Eco RII (CCA/TGG). No discrete bands of DNA were seen with any of these enzymes digestions.

A third group of four enzymes cut less extensively, but produced a regular ladder of low molecular weight bands, based on monomers of about 260 bp. These enzymes were Eco RI (GAATTC), Hind III (AAGCTT), Msp I (CCGG) and Taq I (TCGA). The ladder was particularly clear after Taq I digestion, which left more of the DNA in higher molecular weight classes that did not obscure the lower molecular weight ladder.

Five enzymes produced considerably less cutting than any of the first 12, and two enzymes produced minimal cutting, leaving most of the DNA in high molecular weight fragments. The limited cutting with these enzymes does not appear to be due to incomplete digestion as different batches of these same enzymes give similar results.

Two of the enzymes were tested against methanol-acetic acid fixed, air dried, metaphase chromosome spreads (Fig. 2), using Giemsa staining after enzyme digestion to stain the remaining DNA. Alu I digestion markedly reduced the staining along the chromosomes arms but left intensely stained block of chromatin at the centromeric ends of many chromosomes. This indicates that the tandemly repetitive sequence near the centromere had not been removed and that Alu I site was not present in the tandemly repetitive sequence near the centromere. Hind III, which produced a faint ladder of fragments from

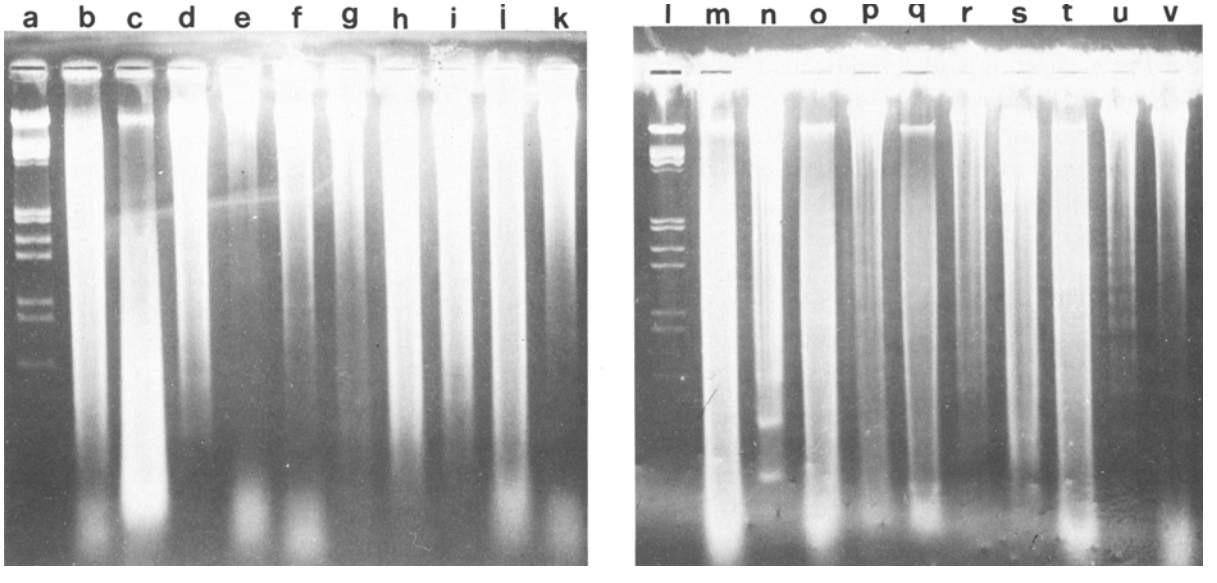

Fig. 1. DNA of *P. sicula* separated by electrophoresis in a 1.2% agarose gel and cleaved with different restriction enzymes: (b) Ava I; (c) Alu I; (d) Bgl II; (e) Cla I; (f) Bam HI; (g) Cfo I; (h) Dra I; (i) Eco RI; (j) Eco RII; (k) Eco RV; (m) Hae III; (n) Hind III; (o) Hinf I; (p) Hpa II; (q) Mbo I; (r) Msp I; (s) Pvu II; (t) Sau3 A; (u) Taq I; (v) Xba I. Molecular size weight marker is phage DNA cut with Hind III/Eco RI (a; l)

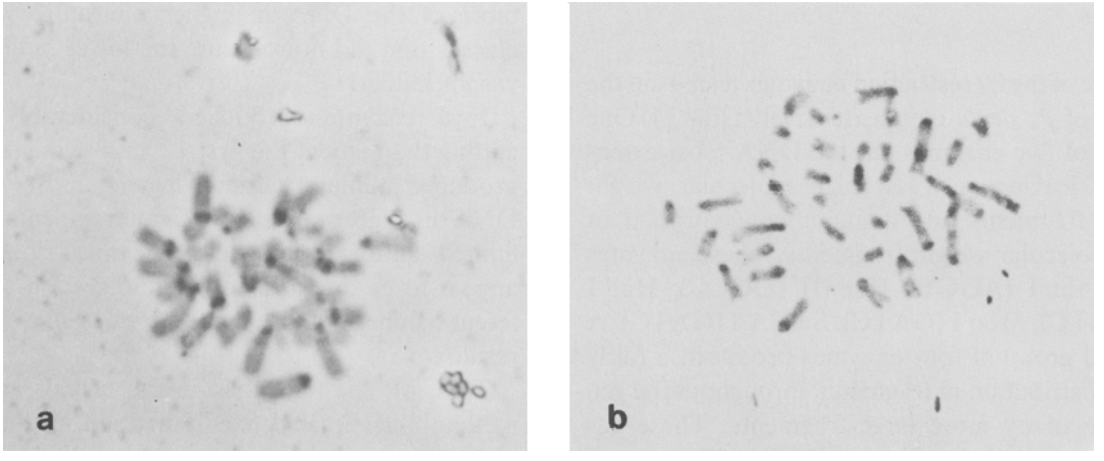

Fig. 2. Cytological preparations from bone marrow of *P. sicula* treated with restriction enzymes: (a) Chromosome plate treated with Alu I; (b) Chromosome plate treated with Hind III.

this repetitive DNA, left more stainable material near some centromeres than along the chromosomes arms.

A typical pattern of regular bands appears after Taq I treatment of *P. sicula* DNA. This indicates the presence in the *P. sicula* genome of a highly repeated satellite like-DNA whose monomer is about 260 bp long (Fig. 3). In order to carry out further studies on this satellite (called pLCSl, Lizard Centromeric Satellite), we extracted the DNA from the above bands and cloned it into pUC 18. We used this as a radioactive probe by nick translation and hybridised it by means of Southern blotting on Taq I digested DNA samples in order to determine the efficiency of our cloning experiment (Fig. 3). Furthermore, *in situ* hybridisation of this probe on metaphase chromosomes of the same species has also been carried out. This experiment clearly shows that the pLCSl is exclusively localized at the centromere of many macrochromosomes at sites corresponding to the C-banding and DAPI staining regions (Fig. 4). It appears probable that this satellite DNA forms part of the highly repeated DNA present in the heterochromatin of this species.

The cloned Taq I DNA fragment was used as probe for dot-blot hybridisation to whole DNA from *P. sicula*, *P. muralis*, *P. wagleriana*, *L. viridis*, *L. dugesii* and *Takydromus sexlineatus*. Results are shown in Figure 5. Under stringent washing conditions (0.1 × SSC, 55 °C) this probe hybridizes with the DNA of all species, except with the DNA of *T. sexlineatus*. Hybridisation intensity decreases with the increase in the phyletic distance from *P. sicula*.

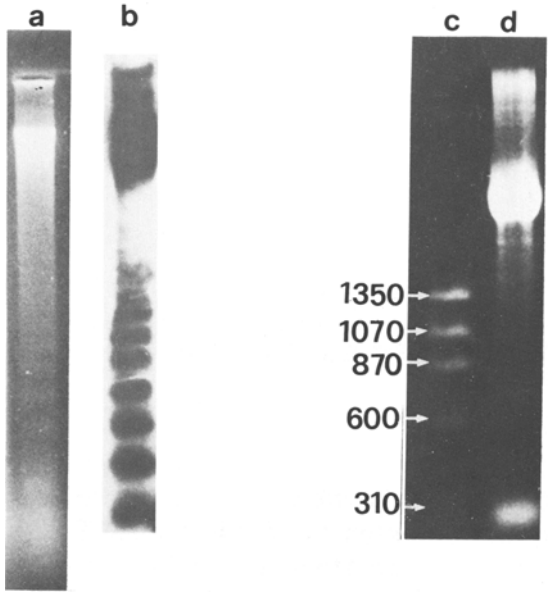

Fig. 3. (a) Agarose gel electrophoresis of Taq I-digested genomic DNA from *P. sicula*; (b) Autoradiography of the same gel, after Southern hybridisation with ^{32}p-pLCSl; (c) X-Hae III Molecular weight standard; (d) Hind III/Eco RI double digested pLCSl.

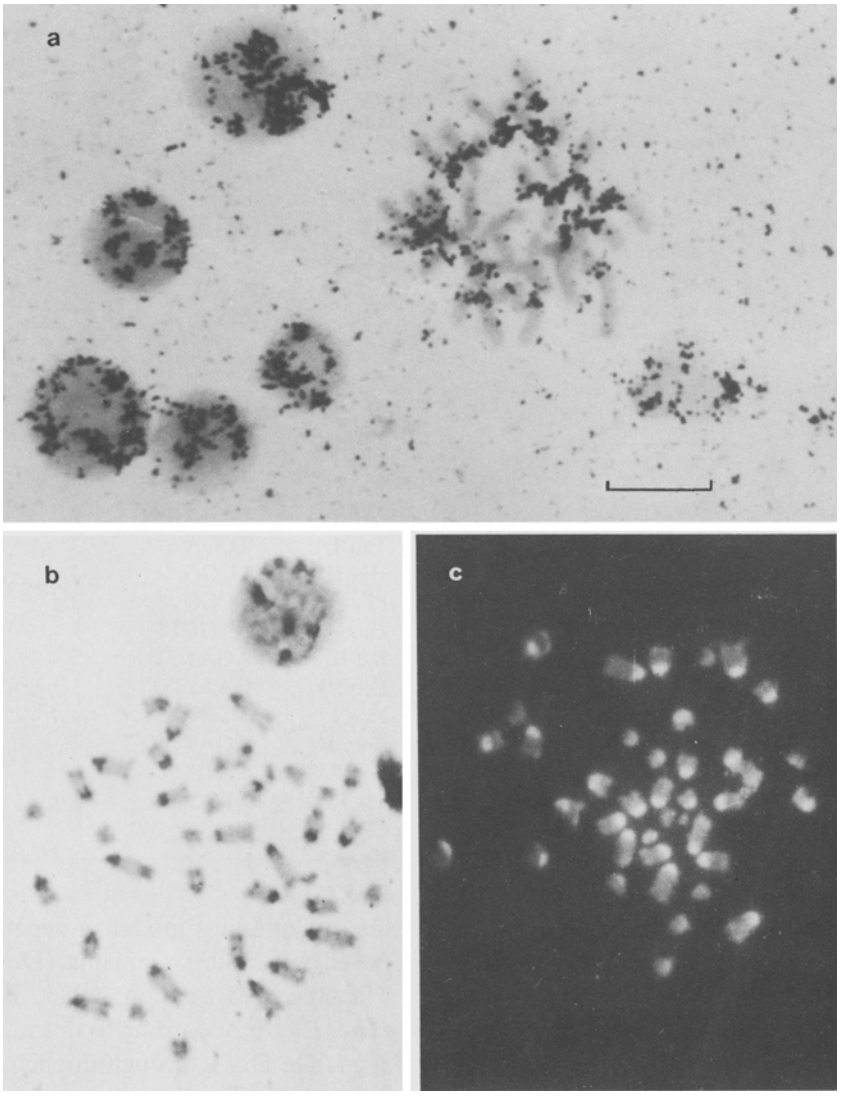

Fig. 4. (a) Autoradiograph of a mitotic metaphase from bone marrow of *P. sicula* after *in-situ* hybridisation with pLCS1 (exposure 18 days). All chromosomes are labelled on the centromere regions. C-banded treated chromosomes of *P. sicula* stained with Giemsa (b) and DAPI (c). Bar = 10 μm.

Discussion

In evaluating the effects of the various restriction endonucleases on the distribution of fragment sizes in *P. sicula* DNA, it is useful to have some estimate of the expected size distribution based on the dinucleotide frequencies in the DNA. Such estimates are readily available only for human DNA (Bishop *et al.*, 1983; Drmanac *et al.*, 1986), which has similar dinucleotide frequencies, including a marked deficiency of the CG dinucleotide (Schwartz *et al.*, 1962). In Table 1, the 23 enzymes have been listed roughly in order of the observed extent of cutting of *P. sicula* DNA, so that by looking at the expected average fragment size one can quickly assess any apparent discrepancies. In general, there is a good correlation between the observed and expected results. One exception, involving Ava I, which cut more than expected, for reasons that remain unclear.

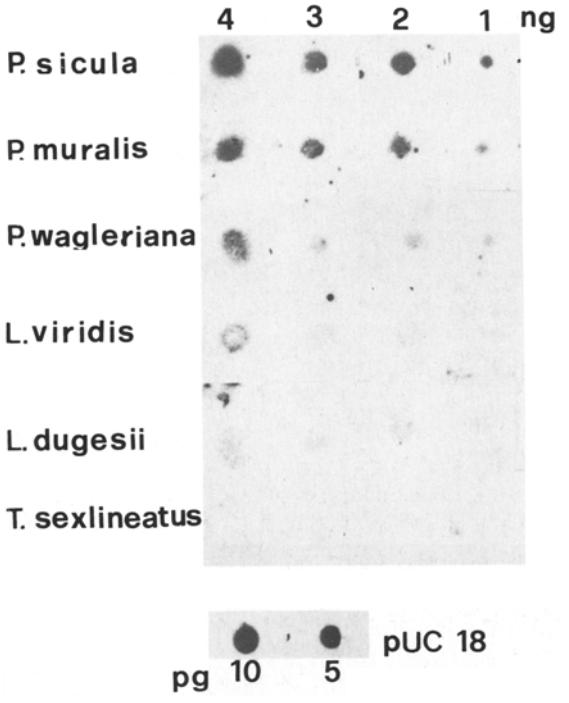

Fig. 5. Dot-blot hybridisation of pLCSl to 4 ng, 3 ng, 2 ng and 1 ng of whole alkali-denaturated DNAs from different species of lacertid lizards. Hybridisation was carried out as described in materials and methods. 10 and 5 pg of pUC 18 DNA were used as control.

Table 1. Relation between the observed degree of cutting by various restriction endonucleases and their expected average fragment sizes.

Enzyme	Recognition site	Extent of cutting in *P. sicula*	Expected fragment size
Alu I	AGCT	1	180
Hinf I	GANTC	1	300
Mbo I	GATC	1	325
Sau3A I	GATC	1	325
Hae III	GGCC A	1	380
Eco RII	CC GG T	2	480
Dra I	TTTAAA	2	1400
Pvu II	CAGCTG	2	3050
Ava I	CPuCGPyG	2	11,700
Taq I	TCGA	3	1100
Hja II	CCGG	3	1650
Msp I	CCGG	3	1650
Hind III	AAGCTT	3	1750
Bgl II	AGATCT	3	2250
Eco RI	GAATTC	3	3200
Xba I	TCTAGA	4	3050
Bam HI	GGATCC	4	5400
Eco RV	GATATC	4	5500
Cla I	ATCGAT	5	15,800
Cfo I	GCGC	5	

Our study indicates that *P. sicula* generally possesses a rather homogeneous genome composition. There is a highly repeated satellite DNA; most readily seen after digestion with Taq I is a ladder of fragments based on a 260 bp monomer. Other ladders are visible though less clearly, with several other enzymes, most notably Hind III.

This satellite is exclusively localized at the centromeric end of many chromosomes.

Our dot blots show that sequences similar to the pLCSl sequence of *P. sicula* are present in other species within the same family including some species (*L. dugesii* and *L. viridis*) which are phylogenetically very distant and have been isolated from *Podarcis sicula* for at least 30 million years (Lutz & Mayer, 1985). This suggests that the Taq I repetitive DNA is relatively old and has become progressively less similar in sequence with the increase in time since the divergence of the various species examined. This is in line with the evolutionary model of repetitive DNA proposed for other organisms (Dover *et al.*, 1982; Macgregor & Session, 1986; Barsacchi Pilone *et al.*, 1986). According to this model the origin of a satellite DNA through duplication processes is followed by a diversification through translocation and gradual degradation.

P. sicula could be a good model to test the validity of this evolutionary trend since it has been found that different subspecies and populations of this species differ quite clearly as to the quantity and localization of constitutive heterochromatin (Olmo *et al.*, 1986, 1987 and unpublished).

An interesting feature of *P. sicula* DNA is the frequency of Dra I sites, TTTAAA. This shows that the genome of *P. sicula* is rather rich in interspersed AT base pair clusters. A more detailed study of this characteristic is in progress. It indicates that interspersed sequences, in *P. sicula* DNA, hybridize extensively with the poly d(AT)

satellite DNA of crab, kindly supplied to us by Dr. Dorothy Skinner (Capriglione *et al.*, unpublished).

In conclusion our preliminary studies supply a certain amount of information on the composition of the *P. sicula* genome and form a starting point for further studies both on DNA evolution in lizards and the variations and properties of particular highly repeated DNA fractions.

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References

Barsacchi-Pilone, G., Batistoni, R., Andronico, F., Vitelli, L. & Nardi, I., 1986. Heterochromatic DNA in Triturus (Amphibia, Urodela) I. A satellite DNA component of the pericentric C-bands. Chromosoma 93: 435–446.

Bernardi, G., Olofsson, B., Filipski, J., Zerial, M., Salinas, J., Cuny, G., Meunier-Rotival, M. & Rodier, F., 1985. The mosaic genome of warm-blooded vertebrates. Science 228: 953–958.

Birstein, V. J., 1982. Structural characteristics of genome organization in amphibians: differential staining of chromosomes and DNA structure. J. mol. Evol. 18: 73–91.

Bishop, T., Williamson, J. A. & Skolnick, M. H., 1983. A model for restriction fragment length distributions. Am. J. Hum. Genet. 35: 795–815.

Denhardt, D. T., 1966. A membrane-filter technique for the detection of complementary DNA. Biochem. Biophys. Res. Comm. 23: 641–646.

Dover, G. A., Brown, S., Coen, E., Dallas, J., Strachan, T. & Trick, M., 1982. The dynamics of genome evolution and species differentiation. In Dover, G. A., Flavell, R. B., (eds) Genome evolution. Academic Press, London, pp. 343–374.

Drmanac, R., Petrovic', N., Glisin, V. & Crkvenjakov, R., 1986. A calculation of fragment lengths obtainable from human DNA with restriction enzymes: an aid for cloning and mapping. Nucl. Acid Res. 14: 4691–4692.

Gall, J. G. & Pardue, M. L., 1971. Nucleic acid hybridization in cytological preparations. Methods Enzymol. XXI D: 470–480.

Jeffreys, A. J. & Flavell, R. A., 1977. A physical map of the DNA flanking the rabbit B-globin gene. Cell 12: 429–439.

Lutz, D. & Mayer, W., 1985. Albumin evolution and its phylogenetic and taxonomic implications in several lacertid lizards. Amph. Rept. 6: 53–61.

Macgregor, H. C. & Session, K., 1986. The biological significance of variation in satellite DNA and heterochromatin in newts of the genus Triturus: an evolutionary perspective. Phil. Trans. R. Soc. Lond. B312: 243–259.

Maniatis, T., Fritsch, E. F. & Sambrook, J., 1982. Molecular cloning. Cold Spring Harbor, New York.

Miller, D. A., Gosden, J. R., Hastie, N. D. & Evans, H. J., 1984. Mechanism of endonuclease banding of chromosomes. Expl Cell. Res. 15: 294–298.

Morescalchi, A. & Olmo, E., 1982. Single copy DNA and vertebrate phylogeny. Cytogenet. Cell Genet. 34: 93–101.

Olmo, E., 1986. A. Reptilia. In: John, B., (ed.). Animal Cytogenetics. 4 Chordata 3. Borntraeger, Berlin-Stuttgart.

Olmo, E., Odierna, G. & Cobror, O., 1986. C-band variability and phylogeny of Lacertidae. Genetica 71: 63–74.

Olmo, E., Odierna, G., Capriglione, T. & Merciai, B. M., 1987. Heterochromatin and genome composition in lacertid lizards. Comp. Biochem. Physiol. 89B No. 1: 1–4.

Schmid, C. W. & Jelinek, W. R., 1982. The Alu family of dispersed repetitives sequences. Science 216: 1065–1070.

Scott, A. F. & Smith, K. D., 1982. Genomic DNA: new approaches to evolutionary sequences in systematic and evolutionary biology. Plenum Press, New York, London. pp. 319–356.

Southern, E. M., 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. mol. Biol. 98: 503–517.

Schwartz, M. N., Trautner, T. A. & Kornberg, A., 1962. Enzymatic synthesis of deoxyribonucleic acid. XI. Further studies on nearest neighbor base sequences in deoxyribonucleic acids. J. Biol. Chem. 237: 864–875.

Thiery, J. P., Macaya, G. & Bernardi, G., 1976. An analysis of eukaryotic genomes by density gradient centrifugation. J. mol. Biol. 108: 219–235.

Ullu, E. & Tschudi, C., 1984. Alu sequences are processed 7SL RNA genes. Nature 312: 171–172.